Anticaries Potential of a Sodium Monofluorophosphate Dentifrice Containing Calcium Sodium Phosphosilicate: Exploratory In Situ Randomised Trial

Short title: Anticaries Potential of a CSPS Dentifrice

C.R. Parkinson[a], M. Siddiqi[a], S. Mason[a], F. Lippert[b], A.T. Hara[b], D.T. Zero[b]
[a]GSK Consumer Healthcare, Weybridge, UK; [b]Oral Health Research Institute, Indiana University School of Dentistry, Indianapolis, IN, USA

Corresponding author: Charles Parkinson, GSK Consumer Healthcare, St George's Avenue, Weybridge, Surrey, KT1 0DE. Email: charles.x.parkinson@gsk.com. Tel: 07920 568 718

Declaration of interests

C.R.P., M.S., and S.M. are employed by GSK Consumer Healthcare. D.T.Z., A.T.H., and F.L. have received compensation from GSK Consumer Healthcare as consultants. This study was supported by GSK Consumer Healthcare.

Key Words

Dentifrice, NovaMin®, Calcium sodium phosphosilicate (CSPS), Fluoride, Caries, In situ model

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Abstract

Calcium sodium phosphosilicate (CSPS) is a bioactive glass material that alleviates dentin hypersensitivity and is postulated to confer remineralization of caries lesions. This single-centre, randomized, single (investigator) blind, placebo-controlled, crossover, in situ study explored whether the addition of 5% CSPS to a nonaqueous, fluoride (F) as sodium monofluorophosphate (SMFP)-containing dentifrice affects its cariostatic ability. Seventy-seven subjects wore four gauze-covered enamel specimens with pre-formed lesions (two surface-softened and two subsurface) placed buccally on their mandibular bilateral dentures for up to 4 weeks. Subjects brushed twice daily with one of the five study dentifrices: 927ppm F/5% CSPS, 927ppm F/0% CSPS, 250ppm F/0% CSPS, 0ppm F/5% CSPS, or 0ppm F/0% CSPS. Specimens were retrieved after either 21 (surface-softened lesions; analyzed by Knoop surface microhardness [SMH]) or 28 days (subsurface lesions; analyzed by transverse microradiography). Enamel fluoride uptake (EFU) was determined on all specimens using a microbiopsy technique. Concentrations of fluoride and calcium in gauze-retrieved plaque were also evaluated. Higher dentifrice fluoride concentrations led to greater remineralization and fluoridation of both lesion types and increased plaque fluoride concentrations. CSPS did not improve the cariostatic properties of SMFP: there were no statistically significant differences between 927ppm F/5% CSPS and 927ppm F/0% CSPS in percent SMH recovery ($p=0.6788$), change in integrated mineral loss ($p=0.5908$) and lesion depth ($p=0.6622$). Likewise, 0ppm F/5% CSPS did not provide any benefits in comparison to 0ppm F/0% CSPS. In conclusion, CSPS does not negatively impact nor does it improve the ability of a SMFP dentifrice to affect remineralization of caries lesions.

Introduction

It is now generally believed that fluoride exerts its anti-caries effects predominantly via relatively small, but protracted, increases in concentration in plaque and saliva [Featherstone, 2008; ten Cate, 2013].To achieve the desired remineralization and anticaries effects of fluoride the dentition should be exposed to elevated levels of fluoride on a continuous basis. The fluoride found within saliva is primarily derived from fluoride-containing dentifrices and although levels of free fluoride initially decrease rapidly after the immediate post-brushing peak, the fluoride clearance profile is believed biphasic with a much slower second phase so that somewhat raised fluoride levels may be found even several hours after brushing [Duckworth et al., 1992]. The net result is that regular use of a fluoridated dentifrice leads to an overall increase in resting levels of fluoride in saliva [Duckworth et al., 1992; Edgar et al., 1992]. However, the availability of calcium and phosphate ions has been reported to be a limiting factor in the retention and prolonged release of fluoride in the oral cavity, and for the net remineralisation of enamel [Cochrane, 2010].

Calcium sodium phosphosilicate (CSPS), a bioactive glass material, was originally developed for the alleviation of dentin hypersensitivity [Gendreau et al., 2011], but may also have potential usefulness for remineralization of caries lesions [Wefel, 2009; Burwell et al., 2009]. The proposed mechanism of action is based on the ability of CSPS to release physiologically relevant levels of calcium and phosphate ions into saliva [Grootveld, 2009] and provide suitable conditions to facilitate the formation of a hydroxycarbonate apatite compound over the surface of dentin [Burwell et al., 2009; Wefel, 2009]. A number of previous studies have tested whether CSPS interacts positively with fluoride in combination dentifrices. Burwell et al. [2009] conducted a series of in vitro studies to investigate the demineralization-prevention and remineralization-enhancement effects of CSPS dentifrices with and without fluoride and observed that these treatments markedly re-hardened enamel specimens. The addition of CSPS as a source of calcium may have been responsible for enhancing the remineralization potential of fluoride in that experimental model. Meanwhile, in a similar preliminary in vitro study, Gjorgievska et al. [2010] reported increased remineralization of enamel with a CSPS-containing dentifrice, as assessed by energy dispersive X-ray spectroscopy analysis, and concluded that this bioactive glass material has potential to remineralize enamel.

This proof-of-principle clinical trial was conducted to elucidate potential interactions of CSPS on the efficacy of a SMFP-containing dentifrice (927 ppm fluoride [F]) to promote remineralization and prevent further demineralization of two lesion types designed to model the earlier and later stages of the caries process—surface-softened lesions and subsurface caries lesions, respectively—using an established in situ caries model [Zero et al., 2004]. Enamel remineralization was assessed by surface microhardness (SMH) for the surface lesions and by transverse microradiography (TMR) for the subsurface lesions. In addition, post-treatment concentrations of fluoride and calcium in the enamel

specimens and plaque from the gauze covering them were evaluated. The 927 ppm F/5% CSPS dentifrice was compared to a 0 ppm F dentifrice with 5% CSPS, and 927 ppm F, 250 ppm F and 0 ppm F dentifrices without CSPS.

Materials and Methods

This was a single centre, randomized, investigator-blind, placebo-controlled, five-treatment, five-period, crossover, in situ study conducted in healthy subjects who provided written informed consent prior to screening. The study was conducted according to the Declaration of Helsinki. This study was funded by GSK Consumer Healthcare and was conducted at the Oral Health Research Institute (OHRI), Indiana University School of Dentistry, Indianapolis, IN, USA with the protocol approved by the IUPUI Institutional Review Board (#1006-65). First enrolment was in July 2010. There were two amendments to the protocol, regarding brushing regimen instructions, specimen randomization, microdrill depth, and wording of analysis instructions.

Study Population

Main inclusion criteria were healthy subjects aged 18–80 years who wore a removable bilateral mandibular partial denture capable of housing specimens and had normal reference range unstimulated and stimulated (by chewing unflavored gum base) salivary flow (pooled saliva ≥0.2 and ≥0.8 mL/minute, respectively). Subjects with active caries lesions or periodontal disease were excluded.

Test and Reference Products and Doses

The following dentifrices were evaluated over a 28-day brushing regimen:

- Experimental: 927 ppm F as SMFP + 5% w/w CSPS (927 ppm F/5% CSPS group)
- Fluoride control: 927 ppm F as SMFP + 0% w/w CSPS (927 ppm F/0% CSPS group)
- Fluoride dose-response control: 250 ppm F as SMFP + 0% w/w CSPS (250 ppm F/0% CSPS group)
- Reference control: 0 ppm F + 5% w/w CSPS (SensiShield™; Periproducts Ltd, UK) (0 ppm F/5% CSPS group)
- Placebo dose-response control: 0 ppm F + 0% w/w CSPS (0 ppm F/0% CSPS group)

With the exception of the Reference control, all the dentifrices were ‘formulation matched’, i.e., the formulations contained the same levels and type of formulation excipients, and none contained any other source of calcium or phosphate beyond CSPS. The Reference control differed from the other formulations with respect to minor differences in the level and type of abrasive silica. The difference in level and grade of silica between the Reference control and the other formulations would not be

expected to impact on the usability of the dentifrice or result in detectable differences in taste by the subject. To maintain investigatorblind masking, all study toothpastes were supplied in plain white tubes.

Clinical Procedures

At screening, subjects were given an oral soft tissue (OST) and oral hard tissue (OHT) examination and their unstimulated and stimulated salivary flow rates were determined. Subjects then entered a ≥6-day washout period during which they followed their usual oral and dental hygiene practices for ≥4 days then returned to the study site for a dental prophylaxis. Thereafter, subjects were instructed to use only the study washout toothpaste (0 ppm F) and toothbrush for 2–3 days before the start of each treatment period. Subjects were provided with a new toothbrush for each new treatment period. Order of treatment was randomly allocated for each subject according to a sequence determined by the Biostatistics Department of GSK Consumer Healthcare.

During each of five 28 day test periods, subjects wore modified bilateral mandibular partial dentures holding four partially demineralized bovine enamel specimens (two with surface-softened and two with subsurface lesions, see below) continuously for 24 hours/day including at mealtimes and while brushing. Subjects could remove the partial denture briefly to rinse their mouth with tap water after eating and to clean the denture. Use of any other dental hygiene products or practices except interdental cleaners such as floss was disallowed during the study period.

The first brushing regimen of each treatment period was completed on site under supervision, following which subjects were instructed to brush their natural teeth only with a full ribbon of study toothpaste at home for 1 timed minute twice daily (morning and at bedtime) for 28 days, taking care not to brush the enamel specimens. The two specimens with surface-softened lesions were removed at the end of 21 days, the two with subsurface lesions were removed at the end of 28 days, having brushed for the final time the night before specimen removal . Compliance with brushing procedures was recorded in subject diaries, as were any new, or changes in existing, medical conditions, medications, or treatments. All subjects received a professional fluoride treatment at the end of the study.

Model Caries Lesions

Specimens were obtained from bovine incisors, polished to create flat surfaces as described elsewhere [Zero et al., 1990]. Surface-softened lesions were created according to a modified method of White [1987]. Enamel specimens were immersed in 40 mL acid buffer (0.05 M lactic acid) 50% saturated with respect to hydroxyapatite with 0.2% (wt/vol) Carbopol® 907 (BF Goodrich, Cleveland, OH, USA) at 37°C for 24 hours. Subsurface lesions were prepared by demineralizing enamel specimens in

8% methylcellulose gel (Sigma M0387, aqueous, 1,500 cP, 63 kDa) covered with an equal mass of 0.1 M lactic acid, adjusted to pH 4.6, at 37°C for 7 days. Lesion quality was deemed acceptable if lesioned areas displayed uniform opacity and surface shine on exposure to overhead light. Following preparation, specimens were stored in a moist environment to prevent dehydration; they were sterilized by ethylene oxide gas prior to insertion into dentures.

A total of four partially demineralized enamel specimens were placed in the buccal flange area on either side of the subject's bilateral partial denture: two 4 × 4 mm enamel specimens with surface-softened lesions and two 4 × 5 mm enamel specimens with subsurface lesions were placed in the buccal flange area on either side of subjects' bilateral partial denture. Pairs of specimens were wrapped together in Polyester Knit Fabric (Item P01628; Bard Peripheral Vascular, Tempe, AZ, USA) to facilitate plaque growth [Koulourides et al., 1974; Featherstone and Zero, 1992] and were mounted flush with the denture surface.

Surface Microhardness

SMH was determined using the Knoop hardness test [Knoop et al. 1939] for specimens with surface-softened lesions using a Wilson 2100 Hardness Tester (Norwood, MA, USA) with indentation length measured using Wilson-Wolpert PC-based Video Filar image analysis software (version 3.5.032) (Illinois Tool Works Inc., Glenview, IL, US). Prior to demineralization, five baseline indentations spaced 100 µm apart were created with a Knoop diamond under a 50 g load. Average indentation length of 43 ±3 µm were deemed acceptable for specimen inclusion. After demineralization, five further indentations were created 100 µm to the left of the baseline indentations and SMH was again determined. Only specimens with indentation lengths 120 ±20 µm were deemed suitable for use. After 21 days' intraoral treatment, five further indentations were made in each specimen to the right of the baseline indentations. The extent of remineralization was calculated as a function of percent reduction of indentation length after versus before in situ intraoral exposure (percent SMH recovery) [SMHR]) [Gelhard et al. 1979].

Transverse Microradiography (TMR)

For specimens with subsurface lesions, changes in integrated mineral loss ($\Delta M=\Delta Z_{base}-\Delta Z_{post}$), lesion depth ($\Delta L=L_{post}-L_{base}$), and maximum mineral density of the lesion surface zone ($\Delta SZ_{max}=SZ_{max,post}-SZ_{max,base}$) before and after treatment were analyzed by TMR. Following lesion creation and after 28 days' treatment, a section approximately 100 µm thick was cut from across the lesion window and sound enamel areas using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications, Lafayette, CO, USA), polished, mounted on plates, and x-rayed at 20 kV and 30 mA at a distance of 42 cm for 65 minutes. Micrographs were examined by Zeiss EOM microscope using TMR software v.3.0.0.11 (Inspektor Research Systems BV, Amsterdam, The Netherlands).

Enamel Fluoride Uptake and Enamel Calcium Uptake

Fluoride and calcium content of partially demineralized enamel specimens were quantified by microdrill enamel biopsy technique [Sakkab et al., 1984]. Briefly, enamel specimens were drilled through the entire lesion in a static-controlled atmosphere to prevent loss of powder due to charging effects (surface-softened lesions, four cores per specimen, 100 µm in depth; subsurface lesions, two cores per specimen each 200 µm in depth). Pooled enamel powder was dissolved in 40 µL 0.5 M $HClO_4$. Half this solution was transferred to tubes containing 1.0 mL $LaCl_3$ and 3.98 mL deonized water and subjected to atomic absorption analysis for calcium content (expressed per unit area enamel cores; µg Ca/cm^2) by Perkin Elmer AAnalyst 200 (Waltham, MA, USA). To the remainder of the solution was added 40 µL citrate/EDTA buffer and 40 µL deionized water; fluoride content was analyzed by fluoride-specific electrode and pH/ion meter and expressed as µg F/cm^2.

Enamel Gauze Plaque

Plaque fluid extracted from gauze strips enclosing enamel samples was analyzed for fluoride and calcium content [Martinez-Mier et al., 2010]. Plaque removed from the gauze was placed in an ultrasonic bath in 200 µL deionized water to create a homogenous sample. For plaque calcium content analysis, 100 µL of the sample was mixed with 900 µL deionized water, 200 µL of 0.01 M NaOH and 100 µL double strength Arsenazo III. Calcium content was obtained by comparing absorbance readings, measured using a spectrophotometer, of calcium standard solutions. For plaque fluoride content, 100 µL of the sample was mixed with 500 µL of deionized water then fluoride was recovered in a 0.05 N NaOH trap solution using a microdiffusion technique [Taves, 1968; Martinez-Mier et al., 2004]. Fluoride content was measured by comparison of the millivolt reading of the sample to standard curves.

Safety

Safety was monitored in terms of adverse events (AEs).

Statistical Analysis

The study aimed to enroll 80 subjects with the intention that approximately 60 subjects would complete the entire crossover study design and provide data for efficacy analysis. With 60 evaluable, completing subjects the study was calculated to have 90% power at the 5% significance level, using two-sided testing, to detect a mean treatment difference for SMHR of approximately 7.6% assuming a within-subject standard deviation of approximately 12.6%.

Safety was analyzed in the Safety population defined as all subjects randomized who received at least one administration of study product. The intent-to-treat (ITT) population was additionally all subjects who provided data for at least one post-baseline efficacy assessment. Efficacy was analyzed in the per protocol (PP) population defined as subjects in the ITT population who had no major protocol violations.

A crossover design was used to eliminate between-subject variability from treatment comparisons, each subject acting as their own control. All specimens were analyzed, however for subjects who did not complete the study; inter-individual differences may not be fully eliminated and may act to decrease the power of the statistical tests for treatment comparisons. For the efficacy parameters SMHR, fluoride or calcium content in enamel, and fluoride or calcium levels in plaque, between treatment analyses were performed using a mixed-model analysis of variance (ANOVA) suitable for crossover studies. The model included a random effect for subject and fixed effects for study period and treatment. For the efficacy parameters, change in mineral content (ΔM), change in lesion depth (ΔL), and change in maximal surface mineralization (ΔSZ_{max}), treatment comparisons were performed using a mixed model analysis of covariance (ANCOVA) model. The model included a random effect for subject and fixed effects for study period and treatment. It also included the corresponding baseline measurement as a covariate. All pairwise treatment comparisons were performed using two-sided testing at the 5% significance level. No adjustment for multiple comparisons was employed as the primary comparison had been defined. The assumptions of normality and homogeneity of variance were investigated. Violations of these assumptions were observed for the variables 'fluoride content of plaque' and 'calcium content of plaque' at both days 21 and 28. These violations were overcome using the log (base 10) transformation.

Results

Study Population

Of 92 subjects screened, 77 subjects (57.1% female; mean age 64.51 [range 30.0–80.0] years) were enrolled and randomized between 21 July 2010 and 17 February 2011. Fifty-one subjects (66.2%) completed the study (Figure 1).

Specimens Analyzed

For the surface-softened lesions (removed after 21 days) all specimens were analyzed. For the sub-surface lesions (removed after 28 days), 21 samples could not be analyzed for reasons including accidental removal on day 21, lost by subject, or subject dropped out of study prior to day 28.

Efficacy

Efficacy results for specimens bearing surface-softened lesions are presented in Table 1 (indentation length data [mean±SD]: sound enamel = 43.2 ± 0.8 µm; lesion baseline = 116.9 ± 9.3 µm). In terms of SMHR, no significant difference was observed following 21 days' treatment between 927 ppm F/5% CSPS and 927 ppm F/0% CSPS, the primary efficacy comparison. Both treatments containing 927 ppm F elicited significantly greater SMHR than those with 250 ppm F/0% CSPS (p=0.0004 for 927/5% CSPS; p=0.0001 for 927/0% CSPS), 0 ppm F/5% CSPS (p<.0001 for both) and 0 ppm F/0% CSPS (p=0.0002 for 927/5% CSPS; p<.0001 for 927/0% CSPS). All other treatment comparisons for SMHR were nonsignificant.

In subsurface lesions (Table 2; lesion baseline data [mean±SD]: ΔZ_{base} = 2533±236 vol%min×µm; L_{base} = 75.3±9.6 µm; $SZ_{max,base}$ = 38.6±6.2 vol%min), after 28 days, while changes in mineral content (ΔM) and lesion depth (ΔL) were not significantly different between the two 927 ppm F dentifrices, both parameters were statistically significantly different in favor of the 927 ppm F dentifrices when either were compared to those containing 0 ppm F, regardless of CSPS content (ΔM: all p<.0001; ΔL: all p<0.02). The 927 ppm F/5% CSPS was also significantly different, in its favor, than the 250 ppm F/0% CSPS dentifrice (ΔM: p=0.0389; ΔL: p=0.0433). ΔSZ_{max} was significantly higher for 927 ppm F/5% CSPS treatment compared with 927 ppm F/0% CSPS (p=0.0114), 0 ppm F/5% CSPS (p=0.0007), and 0 ppm F/0% CSPS (p=0.0370) with no other significant between-treatment differences.
Figure 2 shows mean mineral distribution profiles of subsurface lesions for all post-treatment groups and after initial demineralization (lesion baseline). Mineral gain in the lesion body can be observed for all lesions at the expense of mineral beyond the original lesion. Differences between treatment groups were minimal and largely confined to the extent of secondary mineral loss.

Pairwise treatment comparisons of fluoride content of enamel specimens showed no significant differences between the two 927 ppm F dentifrices or between the two 0 ppm dentifrices following brushing for 21 (surface-softened lesions) or 28 days (subsurface lesions) whereas there were statistically significant differences (all p<.0001) between all higher- versus lower-concentration SMFP formulations, regardless of CSPS content, at both time-points, favoring the higher concentration formulations for all comparisons.

Pairwise treatment comparisons of calcium content of enamel specimens showed no significant difference between the two 927 ppm F dentifrices or between the two 0 ppm dentifrices for either model lesion type. Treatment with 927 ppm F/5% CSPS was associated with significantly higher calcium content than all treatments with lower SMFP content for surface-softened lesions (all p<0.05) and the two treatments with 0 ppm F for subsurface lesions (both p<0.01). There were no other significant between-treatment differences for surface-softened lesions. For subsurface lesions, the

only other statistically significant difference was between 250 ppm F/0% CSPS and 0 ppm F/0% CSPS in favor of the former (p=0.0382).

There was no difference in levels of fluoride in plaque from specimen gauze for surface-softened lesions. For subsurface lesions there was a statistically significantly higher level of fluoride in the 927 ppm F/5% CSPS dentifrice group compared to 927 ppm F/0% CSPS (p=0.0393). Treatment with 927 ppm F with and without 5% CSPS was associated with statistically significantly higher fluoride content of enamel gauze plaque compared with most of the lower-dose SMFP formulations for both lesion types (all $p<0.05$), with the sole exception of the comparison between 927 ppm F/0% CSPS and 250 ppm F/0% CSPS dentifrice in subsurface lesions. No significant difference of gauze plaque calcium concentration was observed between any groups for either lesion type.

Safety

There were a total of 115 treatment-emergent AEs (TEAEs) reported for 52 subjects (67.5%); TEAEs arising in each group are summarized in Table 3. Among these, 54 were oral TEAEs reported in 30 (39.0%) subjects. Five of the TEAEs were deemed treatment-related, all classified as mild: two reports of oral mucosal exfoliation, one in each of the 0 ppm F groups; two reports by one subject of stomatitis in the 0 ppm F/5% CSPS group and one report of dysgeusia by one subject in the 0 ppm F/0% CSPS group. There were six serious AEs (SAEs) observed for five subjects (one case each of pneumonia, large intestine perforation, glioblastoma multiforme, and coronary artery disease and two of unstable angina); none of these SAEs was considered related to study treatment and none led to study discontinuation.

Discussion

It is informative to study the effects of dentifrice in both surface-softened and subsurface lesions as a sensitive experimental model of the caries process [Zero, 1995]. While surface-softened lesions provide insight into the efficacy of anticaries agents during the early stages of caries, surface remineralization of softened enamel lesions may obscure additional mineral loss and/or inhibit deeper mineral deposition beyond the surface layer [Mukai et al., 2001; Lynch et al., 2007; Lynch and Smith, 2012],.The kinetics of enamel demineralization are thought to be surface controlled with a more or less intact (reformed) surface zone being the rate-limiting factor in further pore reduction of the underlying subsurface lesion [Robinson et al., 2000]. Mineral redeposition on the caries lesion surface may derive from material dissolved from deeper layers as well as from plaque fluid that re-precipitates in this region and thereby stabilizes the enamel defect. Fluoride is particularly important in this process because it facilitates redeposition and produces a less acid-soluble mineral [Robinson et al., 2000].

In this study, treatment comparisons were evaluated in the context of a positive fluoride dose response, indicative of model validation [Proskin et al., 1992]. For SMHR, EFU and plaque fluoride, significant differences were observed between the 927 ppm F/0% CSPS and 250 ppm F/0% CSPS dentifrices, in favor of the former. However, for the other efficacy measures, primarily for the subsurface lesions, no significant differences were observed between high and low fluoride treatment groups. Consequently, only limited conclusions can be drawn from differences in these efficacy variables, e.g., while ΔSZ_{max} was statistically significantly higher for 927 ppm F/5% CSPS treatment compared with 927 ppm F/0% CSPS (p=0.0114), in the absence of any fluoride dose response this observation is not considered clinically significant.

The differential behavior of the surface-softened and subsurface lesions was an interesting finding of the present study. Assuming the longer intra-oral exposure of the subsurface lesions was not crucial and that SMH measurements correspond to mineral content [Lippert and Lynch, 2014] and are therefore comparable to the TMR observations on subsurface lesions, it is striking that surface-softened lesions remineralized whereas subsurface lesions demineralized further. These observations are, at least in principle, in disagreement with the present knowledge about the importance of baseline mineral loss of lesions on subsequent in situ de- and remineralization [Strang et al., 1987; Mellberg et al., 1992]. One explanation for the present findings may lie in the fact that lesions were not comparable in that the surface-softened lesions exhibited a different mineral distribution than the subsurface lesions (for comparison, see Lippert and Lynch, 2014 and Lippert et al., 2013). Furthermore, surface layers of both lesion types represent different stages of lesion maturation, a lesion formed over 7 days will present a more defined (but not necessarily more mineralized) surface layer than one that was formed during 24 hours of demineralization. As surface layer formation is a dynamic process, the extended demineralization period will not only affect lesion size but also lesion surface properties that in turn affect its reactivity, porosity and ability to allow in- and outflow of ions.

Within the limitations of this study, the present results indicate that addition of 5% CSPS to SMFP dentifrice neither promotes nor inhibits the ability of fluoride to remineralize enamel. Likewise, it appears 5% CSPS does not offer any benefits in its own right as indicated by results obtained on fluoride-free formulations. A recent study on dentin mineralization [Jones et al., 2015], combined with clinical evidence on the relief from dentin hypersensitivity studies [Gendreau et al., 2011], would suggest CSPS's activity is restricted to dentin and perhaps only to plaque-free surfaces. CSPS has been shown to bind to collagen in dentine [Efflandt, 2002] and precipitate hydroxyapatite-like material onto dentin surfaces [Burwell et al., 2009; Wefel, 2009]. The absence of collagen binding sites on the enamel surface may preclude retention of CSPS to enamel; however, further studies on plaque free enamel surfaces would be required to support this. The results from this study also indicate that CSPS is poorly retained in plaque. No enhancement of calcium in plaque for the CSPS

treatment groups relative to the controls was observed.. Poor retention of CSPS to enamel or plaque surfaces may compromise the ability of CSPS to remineralize caries lesions.

In conclusion, the present in situ caries study found no statistically significant difference in SMHR between the 927 ppm F dentifrices with or without 5% CSPS. These results suggest that the addition of CSPS to a SMFP-containing dentifrice does not negatively or positively impact the ability of fluoride to effect mineralization of surface-softened and subsurface caries lesions. All test dentifrices were generally well tolerated.

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Legends

Figure 1. Trial flow

Figure 2. Mean mineral distribution for baseline lesions (after demineralization) and all post-treatment groups (after in situ phase).

Table 1. Surface-softened lesions: least square means (SE) and statistical analysis of efficacy variables following 21 days' treatment (PP population, n=75 for all)

Table 2. Subsurface lesions: least square means (SE) and statistical analysis of efficacy variables following 28 days' treatment (PP population, n=75 for all)

Table 3. Summary of treatment-emergent adverse events (TEAEs) (Safety population)

Table 1.

Variable	927 ppm F/5% CSPS		927 ppm F/0% CSPS		250 ppm F/0% CSPS		0 ppm F/5% CSPS		0 ppm F/0% CSPS	
SMHR (%)	27.5 (2.19)	A	28.5 (2.31)	A	18.7 (2.20)	B	15.5 (2.30)	B	18.0 (2.23)	B
EFU (µg/cm^2)	19.8 (0.77)	A	19.1 (0.82)	A	12.2 (0.78)	B	7.4 (0.82)	C	7.0 (0.79)	C
ECU (µg/cm^2)	8885.7 (193.55)	A	8617.7 (206.01)	AB	8387.6 (194.80)	B	8369.4 (204.91)	B	8363.4 (198.12)	B
Plaque-F (µg/g)	1.7 (0.06)	A	1.7 (0.07)	A	1.5 (0.06)	B	1.4 (0.07)	B	1.4 (0.06)	B
Plaque-Ca (µg/g)	2.5 (0.06)	A	2.7 (0.06)	A	2.5 (0.06)	A	2.5 (0.06)	A	2.5 (0.06)	A

Statistically significant ($p<0.0005$) within-variable differences highlighted by different letters along rows

SMHR = surface microhardness recovery; EFU = enamel fluoride uptake; ECU = enamel calcium uptake; CSPS = calcium sodium phosphosilicate

Table 2.

Variable	927 ppm F/5% CSPS		927 ppm F/0% CSPS		250 ppm F/0% CSPS		0 ppm F/5% CS
ΔM (vol%·μm)	-474.9 (256.14)	A	-600.8 (267.46)	AB	-946.6 (259.36)	B	-1557.4 (265.33)
ΔL (μm)	36.4 (7.87)	A	39.2 (8.16)	AB	49.1 (7.94)	BC	56.0 (8.11)
ΔSZ_{max} (vol%)	8.2 (0.78)	A	5.6 (0.84)	B	6.5 (0.79)	AB	4.7 (0.83)
EFU (μg/cm^2)	42.3 (1.66)	A	41.5 (1.78)	A	26.0 (1.69)	B	13.3 (1.75)
ECU (μg/cm^2)	15,400.6 (344.04)	A	14,697.3 (370.81)	A	14,899.7 (350.97)	AB	14,176.4 (365.72)
Plaque-F (μg/g)	1.9 (0.07)	A	1.7 (0.07)	B	1.5 (0.07)	BC	1.4 (0.07)
Plaque-Ca (μg/g)	2.4 (0.06)	A	2.5 (0.07)	A	2.4 (0.07)	A	2.4 (0.07)

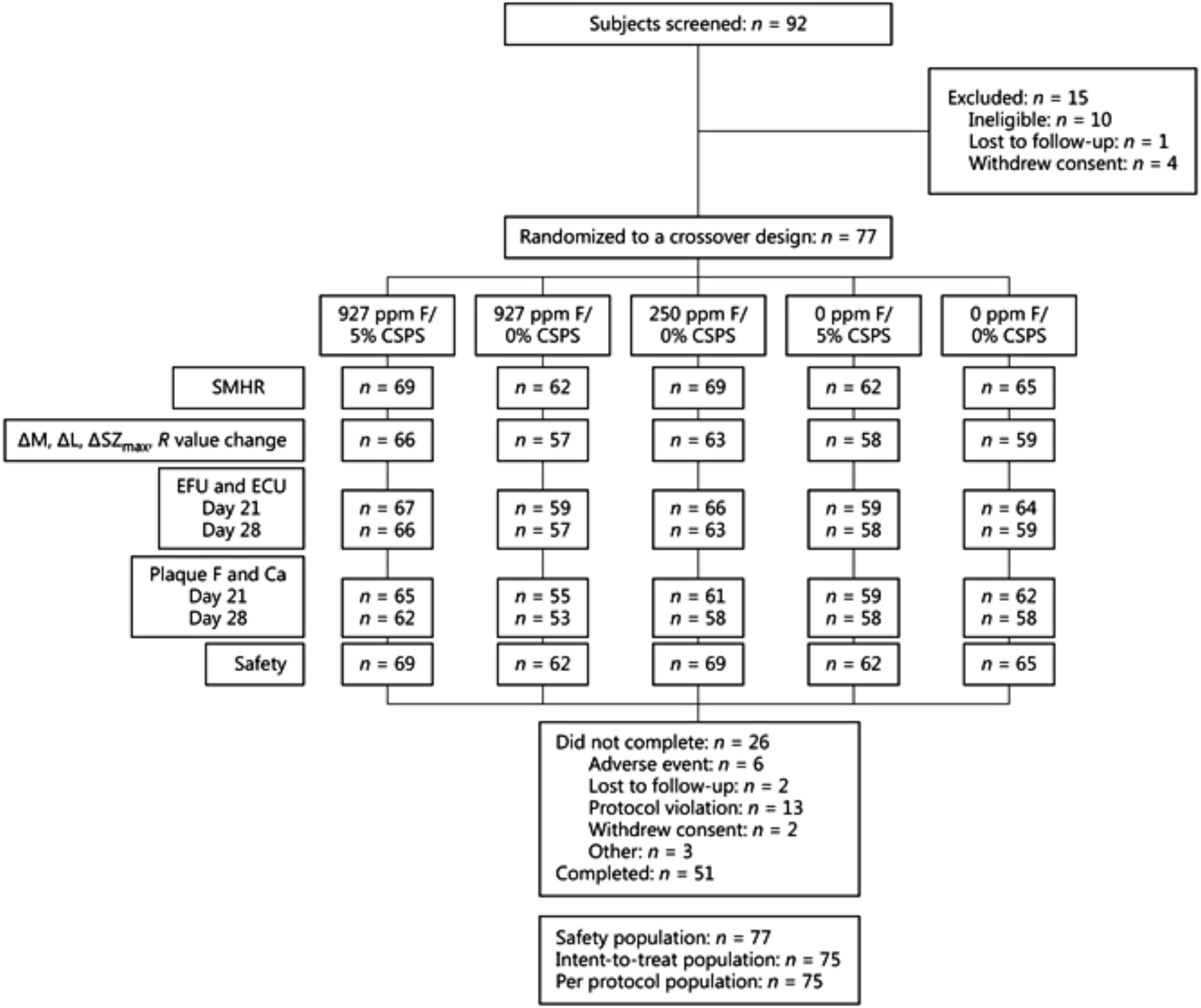
Statistically significant ($p<0.05$) within-variable differences highlighted by different letters along rows

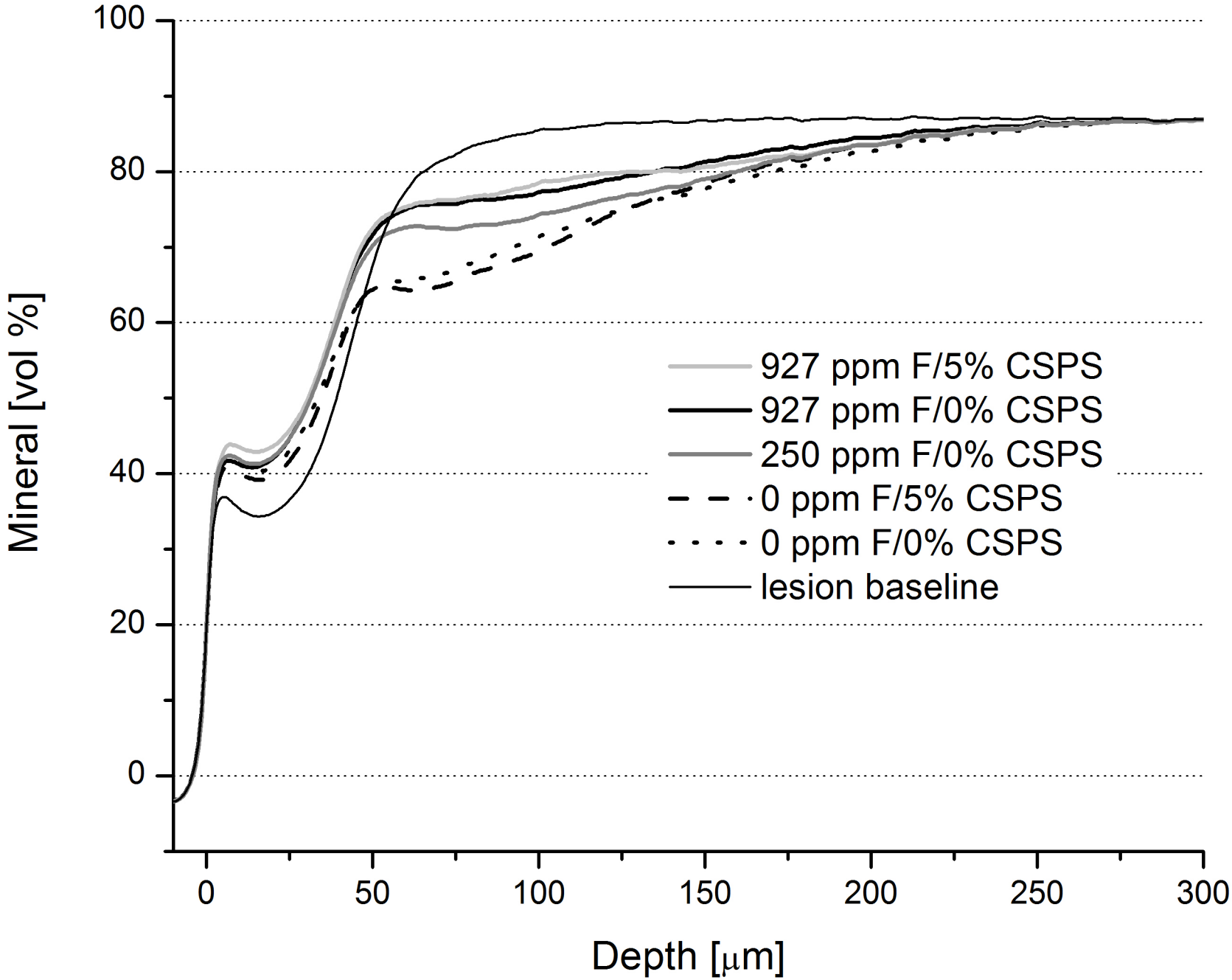
ΔM = change in mineral content; ΔL = change in lesion depth; ΔSZ_{max} = change in maximum mineral density at the surface zone; EFU = enamel fluoride uptake; ECU = enamel calcium uptake; CSPS = calcium sodium phosphosilicate

Table 3.

	927 ppm F/5% CSPS n=69		927 ppm F/0% CSPS n=62		250 ppm F/0% CSPS n=69		0 ppm F/5% CSPS n=62		0 ppm F/0% CSPS n=65	
	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	nAE	n (%)	n
No. subjects with ≥1 TEAE	17 (24.6)	30	9 (14.5)	22	23 (33.3)	27	15 (24.2)	17	14 (21.5)	
Oral TEAE	13 (18.8)	24	3 (4.8)	6	8 (11.6)	9	4 (6.5)	6	5 (7.7)	
Treatment-related TEAE	0	0	0	0	0	0	1 (1.6)	3	2 (3.1)	
Oral mucosal exfoliation	0	0	0	0	0	0	1 (1.6)	1	1 (1.5)	
Stomatitis	0	0	0	0	0	0	1 (1.6)	2	0	
Dysgeusia	0	0	0	0	0	0	0	0	1 (1.5)	

n (%) = number (%) of subjects; nAE = number of AE


Subjects screened: n = 92
Excluded: n = 15
Ineligible: n = 10
Lost to follow-up: n = 1
Withdrew consent: n = 4
Randomized to a crossover design: n = 77
927 ppm F/ 5% CSPS
927 ppm F/ 0% CSPS
250 ppm F/ 0% CSPS
0 ppm F/ 5% CSPS
0 ppm F/ 0% CSPS
SMHR
n = 69
n = 62
n = 69
n = 62
n = 65
ΔM, ΔL, ΔSZmax, R value change
n = 66
n = 57
n = 63
n = 58
n = 59
EFU and ECU
Day 21
Day 28
n = 67
n = 66
n = 59
n = 57
n = 66
n = 63
n = 59
n = 58
n = 64
n = 59
Plaque F and Ca
Day 21
Day 28
n = 65
n = 62
n = 55
n = 53
n = 61
n = 58
n = 59
n = 58
n = 62
n = 58
Safety
n = 69
n = 62
n = 69
n = 62
n = 65
Did not complete: n = 26
Adverse event: n = 6
Lost to follow-up: n = 2
Protocol violation: n = 13
Withdrew consent: n = 2
Other: n = 3
Completed: n = 51
Safety population: n = 77
Intent-to-treat population: n = 75
Per protocol population: n = 75


Mineral [vol %]
100
80
60
40
20
0
0
50
100
150
200
250
300
Depth [μm]
927 ppm F/5% CSPS
927 ppm F/0% CSPS
250 ppm F/0% CSPS
0 ppm F/5% CSPS
0 ppm F/0% CSPS
lesion baseline